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In Re Application of:

KURTZMAN et al.

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Title: METHODS FOR DELIVERING DNA TO THE BLOODSTREAM USING RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS

DECLARATION OF GREGORY M. PODSAKOFF, M.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, GREGORY M. PODSAKOFF, hereby declare as follows:

1. I am a co-inventor of the above-identified application. I hold a Bachelor of Science (B.S.) degree in Biology, a Master of Science (M.S.) degree in Microbiology, and a Doctorate Degree in Medicine (M.D.), from Loma Linda University. I am Board Certified in Internal Medicine. I have practiced internal medicine (in private practice) for over 8 years and conducted research under Postdoctoral fellowships in the fields of Neurobiology and Pediatrics at the City of Hope Medical Center in Duarte, CA.

2. I am currently employed by the Children's Hospital of Los Angeles where I am the Clinical Trial Coordinator of Gene Therapy. I was previously employed by Avigen, Inc., where I was involved in research and development relating to recombinant adeno-associated virus (rAAV)-based gene delivery systems and the transformation of eukaryotic somatic cells therewith. I carried out research and development in recombinant AAV-based technologies at Avigen Inc. from 1995-1998, where I was a Project Manager. I have co-authored numerous scientific publications, as well as numerous abstracts, and particularly I have authored 10 publications relating to

rAAV-based gene delivery. A true and correct copy of my *curriculum vitae* is attached hereto as Exhibit A.

3. I have been involved in research projects pertaining to delivery of a wide variety of genes to the bloodstream using rAAV-based gene delivery techniques. In particular, my co-workers and I have shown successful *in vivo* delivery of rAAV vectors containing various therapeutic genes to the bloodstream, sustained expression of these various genes after delivery to the bloodstream, and therapeutic benefit in treated animals. These results demonstrate the broad applicability of the methodology claimed in the above-identified application.

4. Attached hereto as Exhibit B is a paper I co-authored, Kessler et al., *Proc. Natl. Acad. Sci. USA* (November 1996) 93:14082-14087 ("Kessler"). This paper evidences an *in vivo* therapeutic effect following intravenous delivery of the human erythropoietin (EPO) gene using rAAV-mediated gene delivery techniques. Specifically, rAAV vectors including a gene encoding human EPO were prepared essentially as described at pages 33-35 of the subject patent application. Virions were produced essentially as described at pages 36-37 of the application. Adult female BALB/c mice were injected intravenously with 3×10^{11} viral particles of AAV-EPO, in the tail vein. Blood was obtained from the orbital venous plexus at 20, 41, 62 and 83 days and assayed for EPO expression, and hematocrit levels were assessed. As shown in Table 2 of Kessler, EPO concentrations measured 13 milliunits/ml EPO at 41 days and 21 milliunits/ml EPO at 83 days. Similarly, hematocrit levels rose from 49% to 64% at Day 41 to 75% at Day 83. These data demonstrate that administration of the EPO gene to the bloodstream, using rAAV-based delivery techniques, results in sustained, *in vivo* expression of EPO with a concomitant increase in hematocrit levels, thus evidencing the therapeutic value of this mode of delivery.

5. I have also studied the utility of treating mucopolysaccharidosis type VII (MPS VII), a lysosomal storage disease caused by a genetic deficiency of β-glucuronidase (GUS), using rAAV systems. In particular, Watson et al., *Gene Therapy* (1998) 5:1642-1649, attached as Exhibit C, on which I am a co-author, describes a study where MPS VII mice were injected intravenously, via the tail vein, with 10^{12} AAV-GUS

virus particles. Mice were sacrificed 13 weeks following injection. Untreated MPS VII mice display disease symptoms and accumulate large amounts of glycosaminoglycans in their tissues, leading to severe developmental abnormalites. However, liver tissue from mice intravenously administered AAV-GUS particles was indistinguishable from liver tissue from normal mice. The tissue from treated mice showed no histopathology (see, Figure 2) and normal levels of glycosaminoglycans were present in the liver (see, Table 6). These studies demonstrate that administration of AAV-GUS to the bloodstream produces GUS enzyme for extended periods of time and provides therapeutic benefits.

6. My co-workers and I have also shown that intravenous delivery of rAAV viral particles, encoding ovalbumin (AAV-Ova), induces both humoral and cellular immunity, and, in particular, ovalbumin-specific cytotoxic T lymphocytes (CTLs), *in vivo*. Brockstedt et al., *Clinical Immun.* (1999) 92:67-75, on which I am a co-author and attached as Exhibit D, describes this work. Specifically, rAAV virions, including a gene encoding ovalbumin, were produced essentially as described at pages 36-37 of the application. C57BL/6 female mice were injected intravenously, in the lateral tail vein, with 3×10^{11} AAV-Ova. The mice developed a humoral response to ovalbumin (see, Figure 1). CTLs specific for ovalbumin were isolated from spleens of sacrificed mice 14 days post-injection (see, Figure 2). In order to test the efficacy of this method for inducing protective anti-tumor immunity, the mice were challenged by injection with the melanoma tumor cell line, MO5 20.10, which expresses a H-$2K^b$-restricted ovalbumin-specific CTL epitope. Mice were monitored daily for the appearance of tumors. Tumor development in the AAV-Ova-injected mice was four-fold less than tumor development in mice injected with PBS or a control vector, AAV-lacZ (see, Figure 3). This study demonstrates that delivery of rAAV virions provides therapeutic amounts of expressed tumor antigen to the bloodstream such that tumor development is retarded.

7. Finally, my co-workers have shown that delivery of rAAV virions to the bloodstream, by injection into the portal vasculature, sustains stable expression of the encoded gene. In particular, Nakai et al., *Blood* (1998) 91:4600-4607 ("Nakai"), attached as Exhibit E, injected 6.3×10^{10} rAAV virions, expressing human Factor IX, into the portal vein of adult C57BL/6 mice. Five weeks post-injection, serum human Factor IX

exceeded therapeutic levels, reaching 200 to 320 ng/ml (Figure 5A). Factor IX levels were stable for at least six months (Figure 5B). Immunofluorescent stain of injected mouse liver at 22 weeks demonstrated human Factor IX expression in hepatocytes (Figure 6A). These experiments further demonstrate that delivery of rAAV to the bloodstream provides a therapeutic effect.

8. In light of the information provided above in paragraphs 4-7, it is my opinion that the present application provides sufficient guidance to the ordinarily skilled artisan desirous of practicing the claimed invention. In fact, the above information demonstrates the applicability of our invention to the treatment of several, wholly unrelated disorders, and further demonstrates that rAAV constructs, prepared and delivered according to the methods taught in the patent application, should perform as expected.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 9/17/99

Gregory M. Podsakoff, M.D.